Studies on the Embryology of *Pycnopodia helianthoides* (Brandt) Stimpson

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THE EMBRYONIC DEVELOPMENT of *Pycnopodia helianthoides,* the 20-rayed sea star, which has a small egg (120μ), an indirect form of development, and larval metamorphosis, has not been previously reported in detail. Mortensen (1921) was able to rear only the early gastrula. No other references to the development of *Pycnopodia* have been found. Species of other multi-rayed sea stars with a yolky egg (and in consequence a more direct form of development) are much better known, e.g., *Solaster endeca* (Gemmill, 1912), *Leptasterias hexactis* (Osterud, 1918), and *Crossaster papposus* (Gemmill, 1920).

The main problems requiring study were the developmental stages between Mortensen's gastrula and metamorphosis, and the number of rays formed by the newly metamorphosed sea star. This latter problem arises from Ritter and Crocker (1900), who studied fixed material from the Harriman Alaska Expedition and gave a good description of a series of stages showing ray interpolation starting from a young specimen with eight rays. Nevertheless, in referring to the larval and post larval origin of rays, Ritter and Crocker (1900: 253) "assumed that at least five, probably all, of the *six rays of our youngest specimens* are, as in other starfishes, of larval beginning." The other two rays of their youngest specimen were small and were considered post larval in origin.

The purpose of this study is to report the continuous observation under laboratory culture of the development of *Pycnopodia* from fertilization to completion of metamorphosis. The newly metamorphosed larva has five arms.

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MATERIALS AND METHODS

Adult animals were collected from the intertidal zone on San Juan Island, Washington, and were kept at the Friday Harbor Laboratories in tanks of running sea water. The animals thrived under these conditions, feeding on a variety of mollusks and sea urchins. In order to determine the sex of the animals without sacrificing them, a biopsy was performed by using a hypodermic needle to extract and examine a small amount of gonadal material. The sex and degree of ripeness of the gonad thus determined, a pair of animals was placed in each tank.

On the morning of Apr 8, 1960, the animals in captivity spawned; the male spawned first in each tank. One large female had been observed to spawn earlier during the latter part of Mar. No other spawning occurred although the animals were kept under constant observation. Mortensen (1921) reported the spawning of *Pycnopodia* at Nanaimo, British Columbia, in May and June.

To ensure natural fertilization, gametes were collected with a clean glass pipette as they emerged from the gonopores. They were then washed in filtered sea water. A sperm suspension was added to the eggs and the time of fertilization recorded. The zygotes were cultured in shallow glass dishes containing filtered sea water maintained at a temperature of 10–12 C on shallow sea water tables. The sea water in the dishes was changed daily until the swimming blastula stage was reached. The larvae were fed on a mixture of the solitary diatom *Nitzschia,* and the unicellular alga *Dunaliella.* The older bipin-

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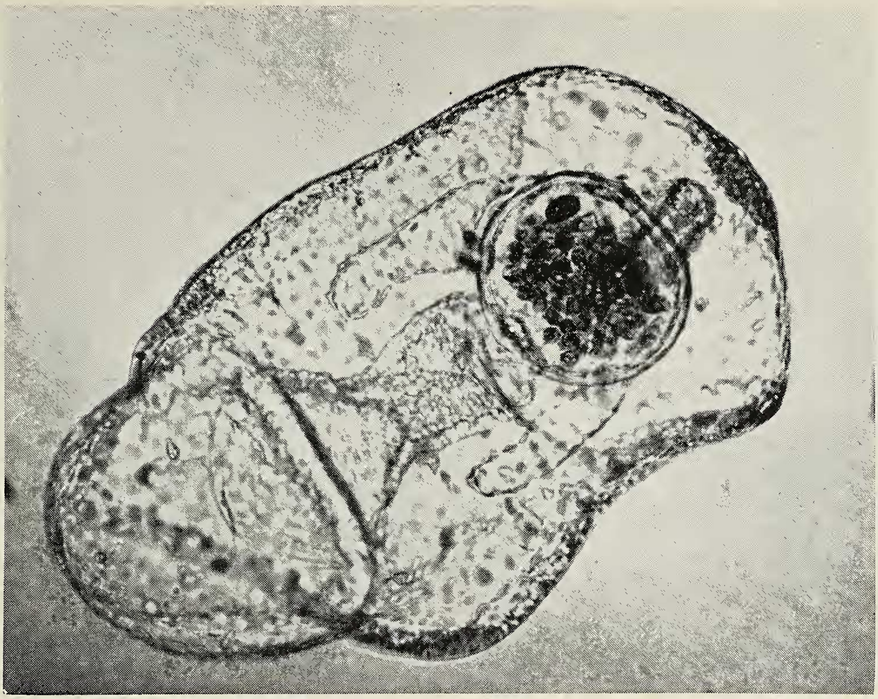

FIG. 1. Photograph of living bipinnarian larva, approximately 23 days old. Dorsal view. Note coelomic sacs and food in stomach. × 200.

narian and brachiolarian larvae were fed additional food materials in the form of marine protozoa and diatoms collected from small tide pools. This variety of food with the occasional supplementation from chance bacterial invasion provided even better results than *Nitzschia* alone. Under these conditions, 12 embryos were reared through metamorphosis. After metamorphosis, the 12 minute sea stars were placed in an aquarium which prevented their escape but allowed a continuous flow of fresh sea water. A variety of food materials was offered to the small sea stars, but none was observed to feed, and no satisfactory food material could be found. Their survival for 5 months after metamorphosis was apparently due to their feeding on debris that accumulated on the bottom of their aquarium.

Samples of the culture were removed for study at intervals throughout the period of development. Early samples were made frequently. They were preserved in Bouin's fixative. Later samples of the large bipinnarian and brachiolarian larvae, taken less frequently, were fixed in 10% formalin in sea water to minimize shrinkage. As it was hoped to follow the growth rate of post-metamorphic specimens for as long as possible and the material was not abundant, only 1 animal out of the 12 was fixed at the time of metamorphosis. Eleven specimens remained with which it was hoped to study growth and further development at least up to Ritter and Crocker's earliest specimens. As explained above, it proved impossible to find the right food. Such growth as did occur over the 5 months of laboratory culture (from approximately 1 mm diameter to 3 mm diameter) could hardly be considered normal. It was noteworthy that there was no further morphological development. Observation during this period had to be restricted to weekends, and the young which died during the interval had dispersed in the culture vessels. Thus there is no fixed material for this phase of the study. The fixed material obtained was stained with Grenacher's Borax Carmine method (Pantin, 1960: 23–24), after which whole mounts were made.

OBSERVATIONS

The embryos within the culture showed a wide variation in size. Even though the exact time of fertilization was known, crowding, feeding, temperature, and other similar factors appeared to influence the growth of the embryos. Despite these variations, the development of *Pycnopodia* closely resembles that of other sea stars having an indirect form of development, e.g., cleavage is holoblastic and almost equal, resulting in a typical blastula and gastrula. Of special interest during the early developmental period, however, is the appearance of large spaces between the blastomeres at the 4-cell stage, causing the blastomeres to appear loosely connected. The blastocoel itself begins to form at the 8- or 16-cell stage. Repeated cell divisions produce a well-formed ciliated blastula which rotates within the fertilization membrane. The blastulae soon "hatch" or break away from this membrane and swim freely. Gastrulation is by embolic invagination. Formation of mesenchyme does not occur until gastrulation is well advanced or completed. The appearance of a mouth completing the digestive system in the bipannarian larva denotes the beginning of feeding (Fig. 1). This stage is reached about 5 days after fertilization. The early development and the features of the larvae resemble those of *Asterias rubens* (Gemmill, 1914); i.e., preoral and post oral ciliated bands, complete digestive system, hydroporic canal and pore development from the left enterocoelic pouch, and the complete and elaborate development of the enterocoelae. The bipinnarian stage is prolonged and the elaborations of the enterocoelae occur late during this stage. The appearance of the brachiolarian arms about the fiftieth day marks the beginning of the brachiolarian larva (Fig. 2). The short brachiolarial arms appear anteriorly surrounding the sucker and provide temporary attachment prior to metamorphosis. Permanent attachment of the larvae is by means of the sucker. Metamorphosis occurs approximately 60–70 days after the onset of development. A summary of the chronological development is given below.

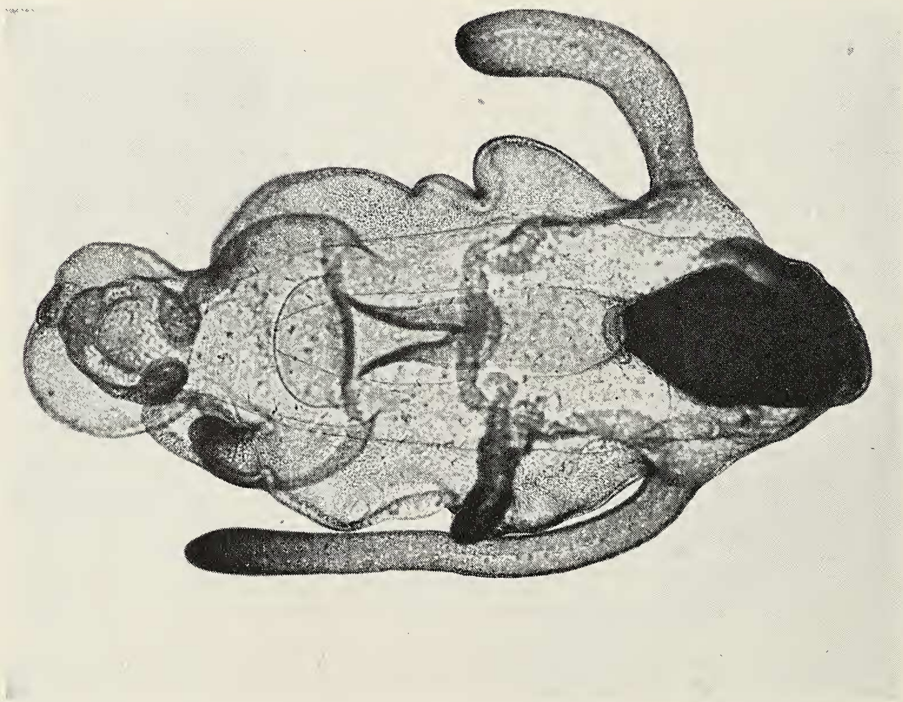

FIG. 2. Photograph of living brachiolarian larva, approximately 7 weeks old. Dorsal view. Note beginning of arm rudiments. $\times$ 55.

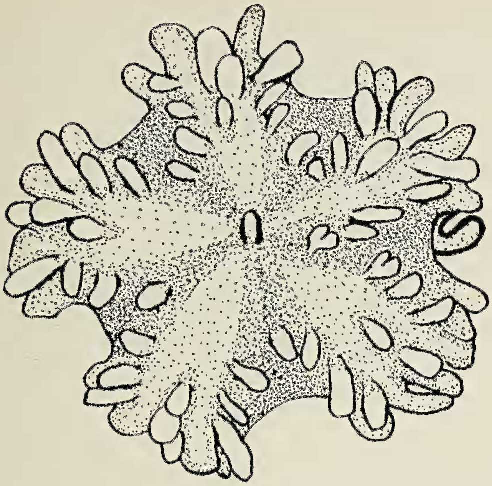

FIG. 3. Aboral view of young 5-rayed *Pycnopodia*. (Camera lucida drawing, × 100.)

1st day: cleavage completed.
2nd day: swimming blastulae.
3rd day: gastrulation completed.
4th day: beginning of mesenchymal formation; formation of enterocoelic sacs.
5th day: early bipinnarian larvae; regions of digestive canal distinct.
6th day: hydroporic canal and pore formed.
12th day: distinct forward and backward extensions of enterocoelic sacs.
32nd day: fusion of right and left anterior coeloms in preoral lobe.
7th week: brachiolarian larvae; formation of brachiolarian arms.
9th week: first metamorphosed animal.
10th week: metamorphosis of all specimens completed.

Shortage of material for sectioning prevented a detailed study of the internal changes during metamorphosis. The development of *Pycnopodia* prior to metamorphosis closely resembles that of *Asterias rubens;* it is therefore probable that this similarity of development also continues through to metamorphosis. Once permanent fixation by larval sucker has occurred, changes in the external morphology of the larva during metamorphosis are rapid. In general, a reabsorption of the larval elements occur, revealing the rudimentary disc and arms of the young sea star. The anterior and middle regions of the larva including "all the larval ciliated processes, as well as the larval anus for the short period during which it can still be recognized, are now on the oral side of the starfish disc" (Gemmill, 1914: 252). The arm rudiments are now clearly visible from the aboral surface of the disc. The young star remains attached by its sucker for 3 or 4 days. When two to three pairs of tube feet have appeared on each arm the sucker is ruptured near the disc and the young sea star begins its free life. The number of arm rudiments clearly visible in this stage of the multi-rayed *Pycnopodia* is *five* (Fig. 3).

The number of primary arms in *Pycnopodia* at the time of metamorphosis has been an open question. Ritter and Crocker's (1900: 260) interpretation of ray multiplication in *Pycnopodia* was made, as they said, "in the absence of knowledge of the embryology of the species." Indeed, no studies of ray formation in other multi-rayed sea stars had been reported at the time. Even though, as reported, *Pycnopodia* is a five-rayed individual at the time of metamorphosis, the question raised by Ritter and Crocker (1900: 260), "What is the relation of ray A [the sixth ray] to the five rays of the asterid ground plan?" still needs an answer. They showed that the sixth ray had a unique relationship to the two bud-

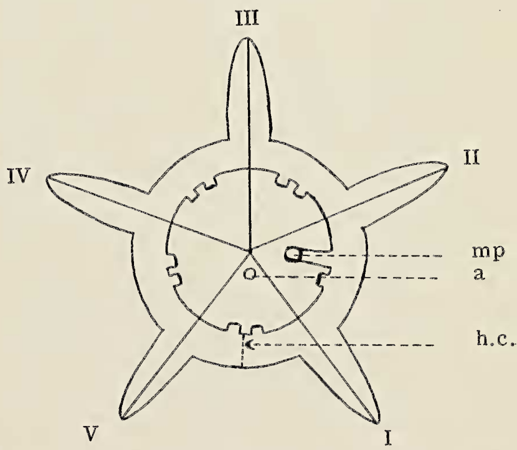

FIG. 4. Aboral view to illustrate numbering of rays and position of hydrocoel closure in 5-rayed asteroids. (Redrawn from Gemmill, 1914.) *mp*, Madreporite; *a*, anus; *h.c.*, hydrocoel closure and position of larval stalk.

ding zones which give rise to all the secondary arms. These secondary arms are interpolated in pairs on each side of the sixth ray. The origin of the sixth ray they supposed to arise from a radial closure of the hydrocoel, i.e., the sixth ray would rise from the exact point of closure. Ritter and Crocker (1900: 265) also suggested "that the two budding zones of the new rays correspond to the region of closure of the hydrocoel ring."

The closure of the hydrocoel ring, so far as is known in five-rayed asteroids, occurs inter-radially (Fig. 4). Information on the embryology of the multi-rayed asteroids *Solaster endeca* (Gemmill, 1912), *Leptasterias hexactis* (Osterud, 1918), and *Crossaster papposus* (Gemmill, 1920) shows that the closure of the hydrocoel is also inter-radial. According to Gemmill (1914), the stalk, hydrocoel closure, and the anus all occur in the same inter-radium in *Asterias*. Therefore, taking the stalk as a point of reference, the hydrocoel closure is inter-radial in *Pycnopodia* (Fig. 5). It is at this point that, if Ritter and Crocker's (1900) hypothesis is true, one would expect to find the sixth ray. However, neither the newly metamorphosed animals nor later forms showed any indication of a sixth ray. The appearance of the sixth ray at this point of closure would still be radial in reference to the hydrocoel itself. Unfortunately, we still have no

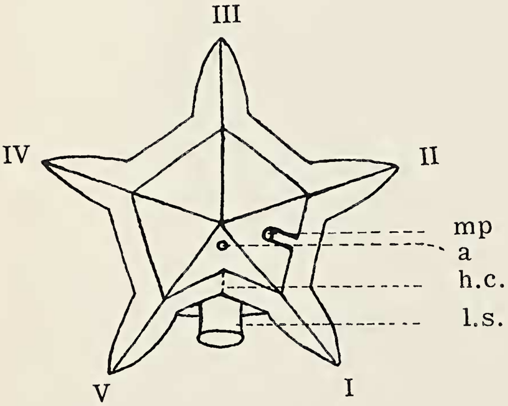

FIG. 5. Aboral view (diagrammatic) to show relationship of hydrocoel closure to larval stalk in *Pycnopodia*. *mp*, Madreporite; *a*, anus; *h.c.*, hydrocoel closure; *l.s.*, larval stalk.

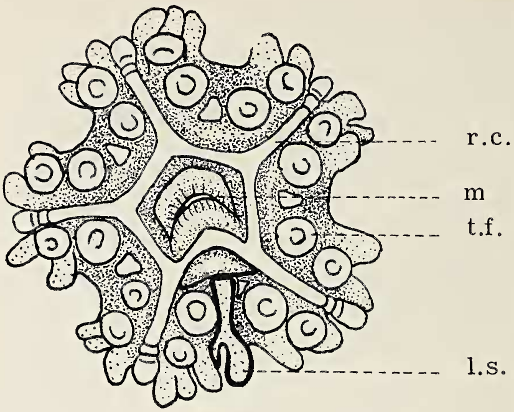

FIG. 6. Oral view of young *Pycnopodia*. Note larval stalk. *l.s.*, Larval stalk; *m*, mouth; *r.c.*, radial canal; *t.f.*, tube feet. (Camera lucida drawing, × 100.)

information of the place and time of origin of the sixth ray. The thickened area shown in oral view in the region of the hydrocoel closure (Fig. 6) is thought to be the disintegrating larval sucker and stalk. The evidence that the "larval organ" in *Pycnopodia* is found at the point of closure is in support of Ritter and Crockers' (1900) suggestion that the sixth ray is in some way possibly related with the "larval organ" of other asteroids.

SUMMARY

This contribution reports that only five primary rays are present in the multi-rayed sea star *Pycnopodia helianthoides* at the time of metamorphosis and suggests that the hydrocoel closure is inter-radial in position. The relationship of the sixth ray to the primary rays and the hydrocoel closure is still unsolved. However, the fact that the five-rayed condition exists for a time after metamorphosis, even though this time may have been lengthened by laboratory culture conditions, demonstrates that the appearance of the sixth ray is secondary to the original five of the asteroid ground plan.

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